

AMENDMENTS TO THE CLAIMS

In the Claims:

Please amend claims 4, 6-9, 11-13, 15-18, and add new claims 22-30 in the following manner. This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Original) A method for the construction of randomized gene libraries in suitable cells comprising the following steps:
 - introducing into cells capable of homologous recombination;
 - a) a target vector comprising a first DNA sequence coding for at least a γ -subunit of a *Kluyveromyces lactis* killer toxin as negative selection marker, said DNA sequence being flanked at its 5' end by a first target sequence and at its 3' end by a second target sequence and;
 - b) a donor DNA sequence which is flanked at its 5' end by a DNA sequence which is homologous to said first target sequence and flanked at its 3' end by a DNA sequence which is homologous to said second target sequence;
 - and
 - cultivation of said cells under suitable conditions allowing the selection of cells in which said DNA sequence in the target vector encoding at least a γ -subunit of a *Kluyveromyces lactis* killer toxin has been replaced by said donor sequence by means of homologous recombination thereby abolishing expression of said γ -subunit of a *K. lactis* killer toxin.

2. (Original) The method of claim 1, wherein said target vector further comprises a second DNA sequence encoding at least one protein region, preferably more than two protein regions, more preferably a full length protein.

3. (Original) The method of claim 2 wherein said first DNA sequence of said target vector encoding at least the γ -subunit of the *K. lactis* killer toxin and being flanked by said two target sequences replaces a protein region encoding DNA sequence of said second DNA sequence comprised in said target vector.

4. (Currently Amended) The method of claim 1 ~~claims 1 to 3~~ wherein said DNA sequence encoding at least the γ subunit of the *K. lactis* killer toxin is under control of a heterologous promoter[[,]] ~~preferably a constitutive promoter, more preferably a TEF promoter from *Ashbya gossypii*.~~

5. (Original) The method of claim 4 wherein said promoter is located between the DNA sequence encoding at least the γ subunit of *K. lactis* killer toxin and one of the two target sequences.

6. (Currently Amended) The method of claim 1 ~~claims 1 to 5~~, wherein said first DNA sequence of said target vector comprises at least one unique recognition site for a restriction enzyme.

7. (Currently Amended) The method of claim 6, wherein said unique recognition site is located in the coding region of the γ -toxin DNA sequence ~~or preferably between the coding region of the γ -toxin DNA sequence and the promoter.~~

8. (Currently Amended) The method of claim 1 ~~claims 1 to 7~~ wherein said second DNA sequence encodes an antibody or a single chain antibody.

9. (Currently Amended) The method of claim 8 wherein said first DNA sequence of said target vector replaces at least one CDR region of said antibody or said single chain antibody[[,]] ~~preferably a CDR3V_L region, more preferably a CDR2 and a CDR3 region.~~

10. (Currently Amended) The method of claims 8 or 9 wherein said first DNA sequence comprising the γ subunit of *K. lactis* killer toxin is transcribed in the opposite direction of said antibody or single chain antibody gene.

11. (Currently Amended) The method of claim 1 ~~claims 1 to 10~~ wherein said γ -toxin subunit of the *K. lactis* killer toxin lacks the signal peptide.

12. (Currently Amended) The method of claim 1 ~~claims 1 to 11~~ wherein said host cells are yeast cells[[,]] ~~preferably *Saccharomyces cerevisiae* cells.~~

13. (Currently Amended) The method of claim 1 ~~claims 1 to 12~~ wherein said target vector is introduced into said host cells in linearized form.

14. (Original) The method of claim 13 wherein said target vector is linearized by cutting with a restriction enzyme recognizing in said first DNA sequence of said target vector said at least one unique recognition site.

15. (Currently Amended) The method of claim 1 ~~claims 1 to 14~~ wherein said donor sequence comprises a DNA sequence encoding a protein region, preferably a CDR region of an antibody.

16. (Currently Amended) The method of claim 1 ~~claims 1 to 15~~ wherein said target vector and said donor sequence are introduced into said host cells by co-transformation.

17. (Currently Amended) The method of claim 12 ~~claims 12 to 16~~ wherein said yeast cells are cultivated at a temperature selected from the range of 24°C to 30°C[[,]] preferably at 24°C.

18. (Currently amended) Use of a *Kluyveromyces lactis* killer toxin[[,]] ~~in particular a γ subunit of said toxin[[,]]~~ as negative selection marker for the construction of randomized gene libraries and region replacement by homologous recombination.

19. (Original) Use of a *Kluyveromyces lactis* killer toxin γ -subunit as negative selection marker for the construction of randomized gene libraries and/or region replacement by homologous recombination.

20. (Original) A DNA vector which comprises the following sequences: a first target sequence for homologous recombination, a *TEF* promoter from *Ashbya gossypii* driving transcription of a *K. lactis* killer toxin, a DNA sequence encoding at least a γ -subunit of a *K. lactis* killer toxin and a second target sequence for homologous recombination.

21. (Currently Amended) A host cell comprising a vector of claim 20[[,]]
~~preferably a yeast cell, more preferably a *Saccharomyces cerevisiae* cell.~~

22. (New) The method of claim 4 wherein the promoter is a constitutive promoter.

23. (New) The method of claim 4 wherein the promoter is a TEF promoter from *Ashbya gossypii*.

24. (New) The method of claim 6 wherein the unique recognition site is located between the coding region of the γ -toxin DNA sequence and the promoter.

25. (New) The method of claim 9 wherein the first DNA sequence of said target vector replaces a CDR3V_L region of said antibody or said single chain antibody:

26. (New) The method of claim 9 where the first DNA sequence of said target vector replaces a CDR2 and a CDR3 region at said antibody or said single chain antibody.

27. (New) The method of claim 12 wherein said host cells are *Saccharomyces cerevisiae* cells.

28. (New) The method of claim 15 wherein said donor sequence comprises a DNA sequence encoding a CDR region of an antibody.

29. (New) The host cell of claim 20 which is a yeast cell.

30. (New) The host cell of claim 29 which is a *Saccharomyces cerevisiae* cell.